Inhibition of endothelial cell migration by cerivastatin, an HMG-CoA reductase inhibitor: contribution to its anti-angiogenic effect

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Received 11 January 2001; revised 26 February 2001; accepted 13 March 2001

First published online 10 April 2001

Edited by Masayuki Miyasaka

Abstract Recent studies have suggested that inhibitors of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (statins) can play a role in protection against vascular risk, which is independent of cholesterol reduction. It could act by inhibiting the synthesis of isoprenoids (farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP)), which are respectively essential for membrane attachment and biological activity of GTPases Ras and RhoA. This study demonstrates that a statin (cerivastatin) inhibits angiogenesis. This effect was due to a decrease in endothelial cell locomotion which was reversed by GGPP. It was mainly related to delocalization of RhoA from cell membrane to cytoplasm, responsible for the disorganization of actin stress fibers. Furthermore, a decrease in MMP-2 secretion, involved in cell invasion, was also observed. This effect is rather due to Ras inhibition as it was reversed by FPP. This anti-angiogenic activity could explain the beneficial effect of statins on atherosclerosis and on cancer prevention as shown by clinical studies.

Key words: Anti-angiogenesis; Migration; HMG-CoA reductase inhibitor; Cerivastatin; RhoA; Ras

1. Introduction

Angiogenesis is characterized by the formation of new capillaries from pre-existing vessels. This event is a prerequisite for both physiological and pathological processes as previously reported [1]. The poor prognosis of some diseases like cancer has been shown to correlate with an increase in angiogenesis. An excessive vascularization can also contribute to other pathological phenomena such as atherosclerosis plaque formation and chronic inflammation [2].

Angiogenesis is a process induced by angiogenic factors. Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) were the two most well-recognized angiogenic factors. Recently, monocyte-secreted cytokine oncostatin M (OSM) was identified as another potent angiogenesis stimulating factor which could play a major role in the development and complication of atherosclerosis [3]. These factors contribute in two crucial steps of angiogenesis, i.e. endothelial cell proliferation and migration. Besides these cytokines, various serine proteases such as urokinase type plasminogen activator and plasmin as well as matrix metalloproteinases (MMPs) are also implicated in the cell migration process [4]. Angiogenesis can be inhibited by anti-angiogenic factors. Various anti-angiogenic factors so far identified like angiostatin, endostatin and thrombospondin are all protein fragments [5]. These raise the problem for pharmaceutical production and the cost expense for long term therapeutically administration required by anti-angiogenic therapy. Some small anti-angiogenic molecules like marimastat exhibit considerable side effects in the clinical assay [6]. So, the development of new anti-angiogenic factors appears emergent for both anti-cancer and anti-atherosclerosis therapies.

The 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, cerivastatin, is initially known to inhibit cholesterol biosynthesis. Recent reports showed that cerivastatin has pleiotropic effects including the inhibition of smooth muscle cell migration and proliferation [7]. Recently, it was also shown that statins act on endothelial cells, as reported by Mussoni et al., fluvastatin inhibits the synthesis of plasminogen activator inhibitor (PAI-1) and induces the secretion of tissue plasminogen activator suggesting an improvement in the fibrinolytic pathway [8].

In fact, the inhibition of HMG-CoA reductase by statins leads to a decreased synthesis of cholesterol and also its precursors, which are isoprenoid products of mevalonate (MVA). These isoprenoids, farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP), provide lipophilic anchors which are essential for membrane attachment and biological activity of small GTP binding protein from the Ras family [9]. For exerting their role in cell signal transduction, protein Ras and RhoA of the GTPase family must translocate from the cytoplasm to the cell membrane. This translocation requires FPP for Ras and GGPP for RhoA [10]. Activation of Ras is involved in the activation of mitogen-activated protein kinase and nuclear factor-kappa B (NF-κB) pathways [11] which can play a pivotal role in angiogenesis [12–15]. Activated RhoA is known to associate with cortical actin in focal contact sites at

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Abbreviations: HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; MMP, matrix metalloproteinase; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; OSM, oncostatin M; MVA, mevalonate; FPP, farnesylpyrophosphate; GGPP, geranylgeranylpyrophosphate; HMEC, human microvascular endothelial cell(s); FCS, fetal calf serum; PBS, phosphate-buffered saline; RT-PCR, reverse transcriptase-polymerase chain reaction

0014-5793/01/$20.00
PII: S0014-5793(01)02337-7

cell membrane ruffles, and therefore is crucial for the organization of actin cytoskeleton and as consequence for cell locomotion which is of prime importance in angiogenesis [16]. Furthermore, the use of the exoenzyme, clostridium botulinum C3 transferase, which specifically prevents the activation of Rho GTPase, inhibits angiogenesis in vitro and in vivo [17].

Because cerivastatin inhibits FPP and GGPP biosynthesis by inhibiting HMG-CoA reductase, we were prompted to analyze the consequence of such inhibition on endothelial cell migration and angiogenesis.

In this study, we demonstrate that cerivastatin inhibits the migration of endothelial cells and the capillary tube formation stimulated by angiogenic factors, i.e. bFGF, VEGF and OSM. We tested OSM in addition to well-known angiogenic factors (bFGF and VEGF) because this inflammatory cytokine is largely expressed in the atheromatous plaque. We also assessed the molecular mechanism of such inhibition related particularly to Ras and RhoA inhibition.

2. Materials and methods

2.1. Cytokines and cerivastatin

R&D Systems (Minneapolis, MN, USA) supplied recombinant human OSM, VEGF and bFGF. Cerivastatin was kindly provided by Bayer-Pharma (Puteaux, France).

2.2. Cell culture

The HMEC-1 (human microvascular endothelial cell) cell line was provided by Dr. Ades (Centers for Disease Control and Prevention, Atlanta, GA, USA) [18]. HMEC-1 were cultured in MCDB-131 medium (Sigma, St. Louis, MO, USA), supplemented with 15% fetal calf serum (FCS), 100 IU/ml penicillin, 100 μg/ml streptomycin, 10 ng/ml epidermal growth factor (Euromedex, Souffelweyersheim, France) and 1 mg/ml hydrocortisone (Pharmacia-Upjohn, Saint Quentin en Yvelines, France).

2.3. Cell migration assays

2.3.1. By transwell method. HMEC-1 were detached with EDTA 0.5 mM, washed twice in phosphate-buffered saline (PBS) and resuspended in MCDB-131 medium with 0.2 mg/ml bovine serum albumin (BSA, Sigma). 50×10^3 cells were seeded in the upper chamber of a transwell insert (PTFE membrane with 8 μm diameter pores, Dutcher, Brumath, France). The lower chamber was filled with 1 ml of MCDB-131 with 2 mg/ml of BSA without or with angiogenic factors (bFGF, VEGF or OSM) used at indicated concentrations. In order to test the effect of HMG-CoA reductase inhibitor on cytokine-induced chemotaxis, cerivastatin was added to the upper chamber at a final concentration of 10 and 25 ng/ml. After 24 h, migrated cells were scraped from the lower surface of the membrane with a cell scraper (Nunc) and then suspended in the medium of the lower chamber to count all migrating cells (both adherent and cells in suspension). These cells were counted with a hemocytometer (Coulter Z1, Coultronics). To address whether inhibition of isoprenoid intermediates of cholesterol biosynthesis is involved in the cerivastatin effect, experiments were performed in presence of MVA (100 μM), FPP (10 μM) or GGPP (10 μM) (Sigma).

2.3.2. By wound healing method. Endothelial cells were cultured in 24-well culture plate. When HMEC-1 were confluent, a wound was performed under standard conditions. Then after washing with PBS, the cells were incubated for 24 h with MCDB-131 containing 2% FCS (concentration of FCS which allows cell survival but not cell proliferation) without or with growth factors (bFGF, VEGF or OSM) used at indicated concentrations. All the assays were performed in the absence or presence of cerivastatin at indicated concentrations. Experiments were performed with and without MVA, FPP or GGPP as indicated above. After a 24 h incubation, cells were washed twice with PBS and then fixed in 4% paraformaldehyde in PBS for 10 min at room temperature. The cells were then stained with Giemsa. Cells migrated into the wound site were photographed at a magnification of 50×.

2.4. Capillary tube formation from microcarriers in three-dimensional fibrin gel

The capillary tube formation assay was performed by the technique of Nehls et al., slightly modified [19]. Formation of capillary tube arising from the periphery of microcarrier beads was observed and photographed with a camera on a reverse microscope at the 4th day of culture.

2.5. Confocal microscopy analysis of RhoA and actin filaments

The confocal microscopy analysis of RhoA and actin filaments was performed, according to the protocol of Menager et al. [16], on the bFGF-stimulated HMEC-1 after an 18 h incubation with cerivastatin. RhoA was detected using first a monoclonal antibody against RhoA (Santa Cruz Biotechnology, CA, USA) and second a fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Immunotech). Actin filaments were visualized by tetra methyl rhodamine isothiocyanate (TRITC)-labeled phalloidin. Computer-assisted image analysis of fluorescence was performed using a confocal microscopy scanning laser microscope (Leica TCS, wavelength excitation 488 nm, emission 525 nm for FITC; 540/570 nm for TRITC).

2.6. MMP-2 secretion

2.6.1. By reverse transcriptase-polymerase chain reaction assay (RT-PCR). To isolate RNA, cells were incubated in a 6-well plate (Nunc) up to confluence and then incubated for 6 h with or without the cytokines and cerivastatin. Cells were then detached by a non-enzymatic cell dissociation solution (Sigma) and washed twice in PBS. Total RNA extraction was performed using SV total isolation system (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Table 1
Inhibitory effect induced by cerivastatin on OSM-, bFGF- and VEGF-stimulated HMEC-1 migration by transwell method after a 24 h incubation

	Control	OSM	bFGF	VEGF
Without cerivastatin	1	2.06 ± 0.06*	1.46 ± 0.04*	1.40 ± 0.03*
Cerivastatin 10 ng/ml	1.03 ± 0.05	1.22 ± 0.04***	1.09 ± 0.06**	1.16 ± 0.03**
Cerivastatin 25 ng/ml	0.98 ± 0.06	0.94 ± 0.03***	1.08 ± 0.02***	1.05 ± 0.04***
MVA	1	1.95 ± 0.06*	n.d.	n.d.
Cerivastatin 10 ng/ml+MVA	1.01 ± 0.06	1.92 ± 0.03*	n.d.	n.d.
Cerivastatin 25 ng/ml+MVA	1.05 ± 0.05	1.89 ± 0.05*	n.d.	n.d.
FPP	1	2.03 ± 0.08*	n.d.	n.d.
Cerivastatin 10 ng/ml+FPP	1.02 ± 0.06	1.34 ± 0.05***	n.d.	n.d.
Cerivastatin 25 ng/ml+FPP	1.03 ± 0.03	1.08 ± 0.04***	n.d.	n.d.
GGPP	1	1.97 ± 0.08*	1.46 ± 0.04*	1.36 ± 0.04*
Cerivastatin 10 ng/ml+GGPP	1.12 ± 0.03	1.88 ± 0.08*	1.56 ± 0.07*	1.30 ± 0.06*
Cerivastatin 25 ng/ml+GGPP	0.97 ± 0.04	1.94 ± 0.04*	1.37 ± 0.05*	1.33 ± 0.05*

Effect of MVA, FPP and GGPP on cerivastatin-induced inhibition of endothelial cell migration.
Results of four experiments in duplicate are expressed as the ratio of the number of migrated cells over the number of migrated cells in each control ± S.E.M. (*$P<0.001$ as compared with cerivastatin-untreated cells, **$P<0.05$; ***$P<0.001$ as compared with OSM-, bFGF- or VEGF-treated cells, $n=4$). MVA = 100 μM; GGPP = 10 μM; FPP = 10 μM (n.d., not determined).

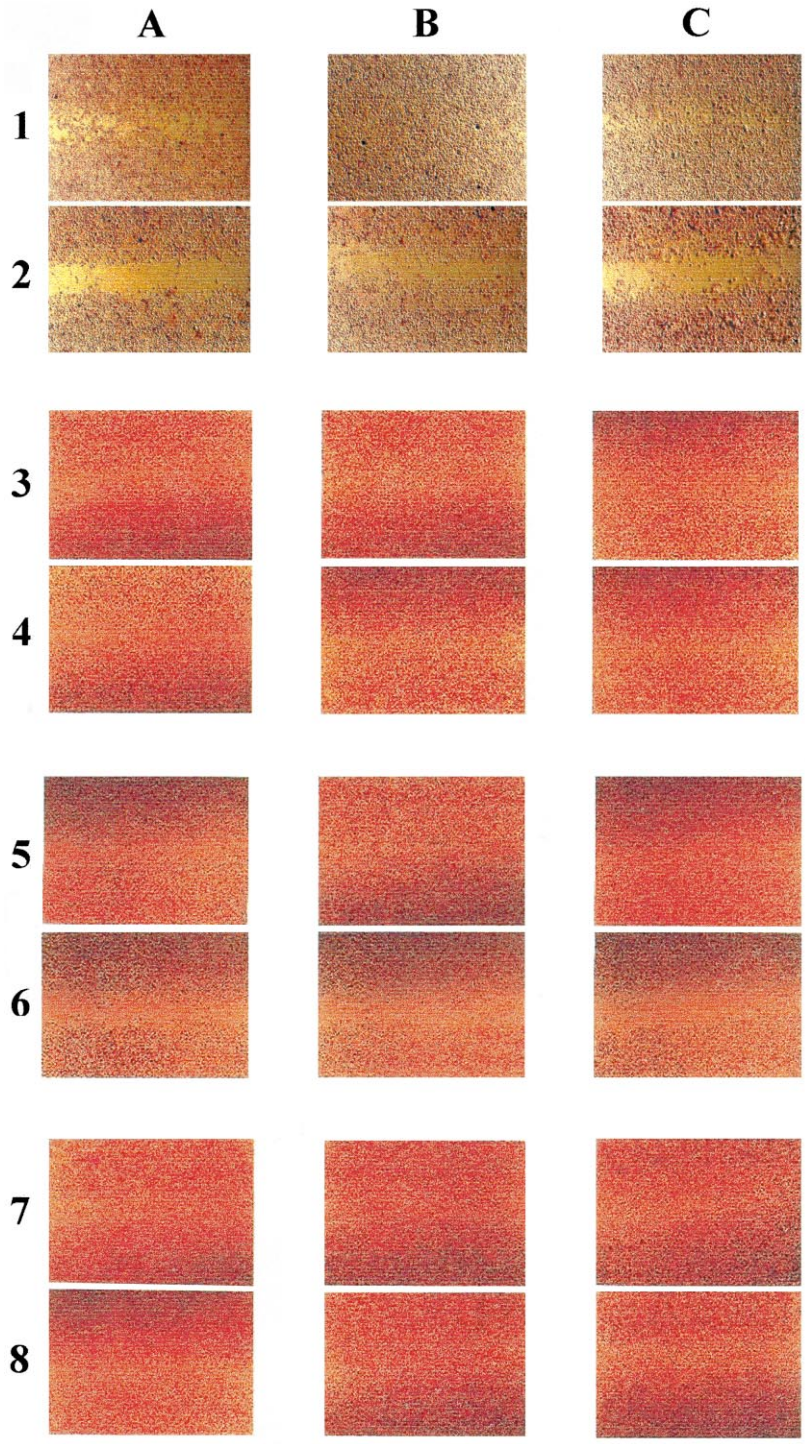

Fig. 1. Inhibitory effect induced by cerivastatin on OSM- and bFGF-stimulated HMEC-1 migration by wound healing method after a 24 h incubation. Effect of MVA, FPP and GGPP on cerivastatin-induced inhibition of endothelial cell migration. Column A: unstimulated cells, column B: OSM 2.5 ng/ml, column C: bFGF 25 ng/ml. Line 1: control, line 2: cerivastatin 25 ng/ml, line 3: MVA 100 μM, line 4: MVA 100 μM+cerivastatin 25 ng/ml, line 5: FPP 10 μM, line 6: FPP 10 μM+cerivastatin 25 ng/ml, line 7: GGPP 10 μM, line 8: GGPP 10 μM+cerivastatin 25 ng/ml.

For RT-PCR, oligonucleotide primers were chosen using a biomolecular sequence databases (GenBank) and were synthesized by Genset (Paris, France). The sequences of MMP-2 sense primer (5′-GGCCCTGTCACTCCTGAGAT-3′) and MMP-2 anti-sense primer (5′-GGCATCCAGGTTATCGGGGA-3′) or β-actin sense primer (5′-ATCTGGCACCACACCTTCTACAATGAGCTGCG-3′) and β-actin anti-sense primer (5′-CGTCATACTCCTGCTTGCTGATCCAC-ATCTGC-3′) were used in a RT-PCR assay. RT-PCRs were performed in the same condition as described previously [20]. The MMP-2 and the β-actin mRNA amplification product were size-fractionated through a 1.5% agarose gel electrophoresis using ethidium bromide staining.

2.6.2. By sodium dodecyl phosphate (SDS)–polyacrylamide gel electrophoresis zymography for MMP assay. The supernatants of HMEC-1 incubated for 24 h, in the absence or in the presence of cerivastatin with or without MVA, FPP or GGPP, were collected.

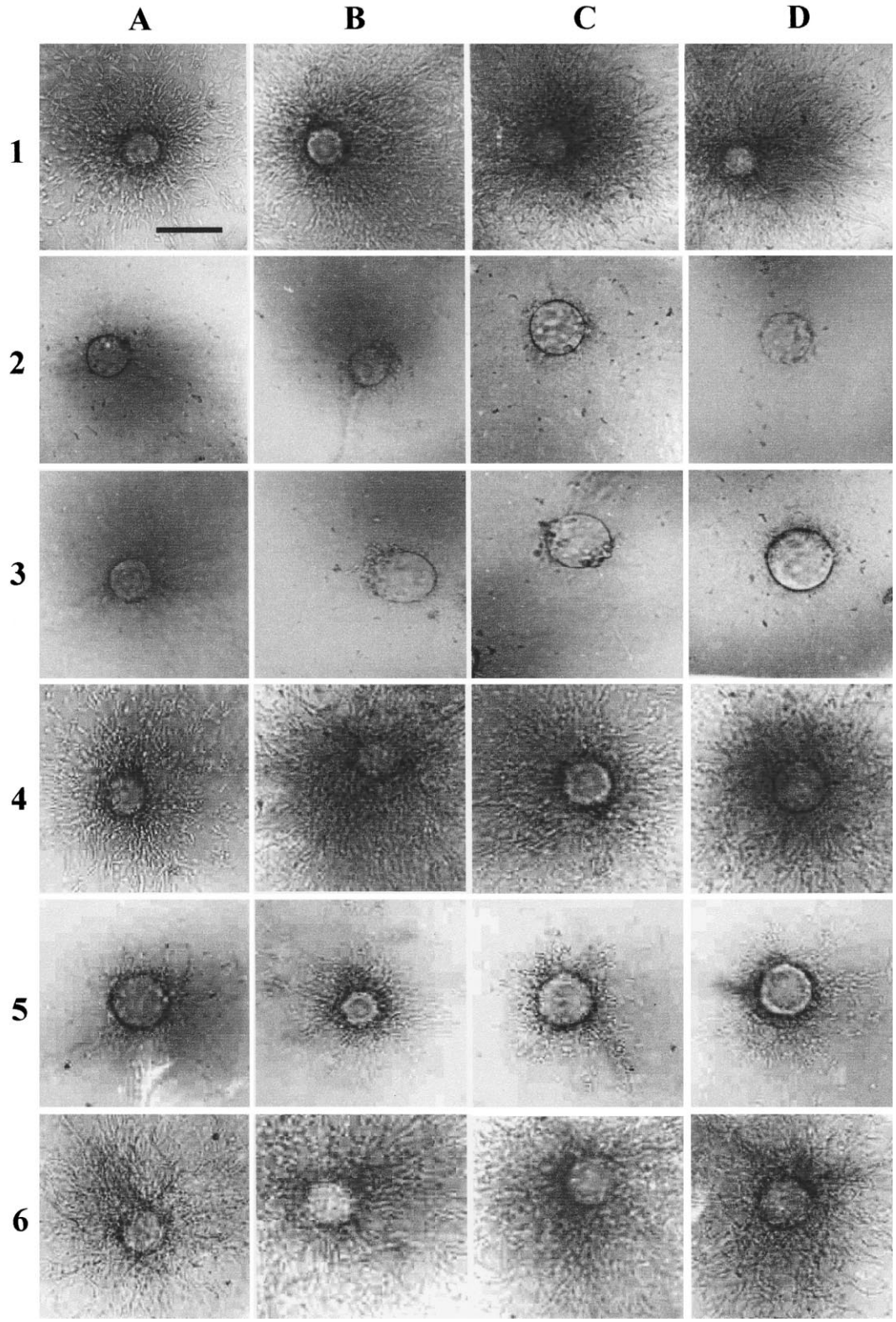

Fig. 2. Inhibitory effect induced by cerivastatin on OSM-, bFGF- and VEGF-stimulated capillary tube formation in a three-dimensional fibrin gel. Effect of MVA, FPP and GGPP on cerivastatin-induced inhibition of capillary tube formation. Column A: unstimulated cells, column B: OSM 2.5 ng/ml, column C: bFGF 25 ng/ml, column D: VEGF 20 ng/ml. Line 1: control, line 2: cerivastatin 10 ng/ml, line 3: cerivastatin 25 ng/ml, line 4: MVA 100 μM+cerivastatin 25 ng/ml, line 5: FPP 10 μM+cerivastatin 25 ng/ml, line 6: GGPP 10 μM+cerivastatin 25 ng/ml (scale bar = 150 μm).

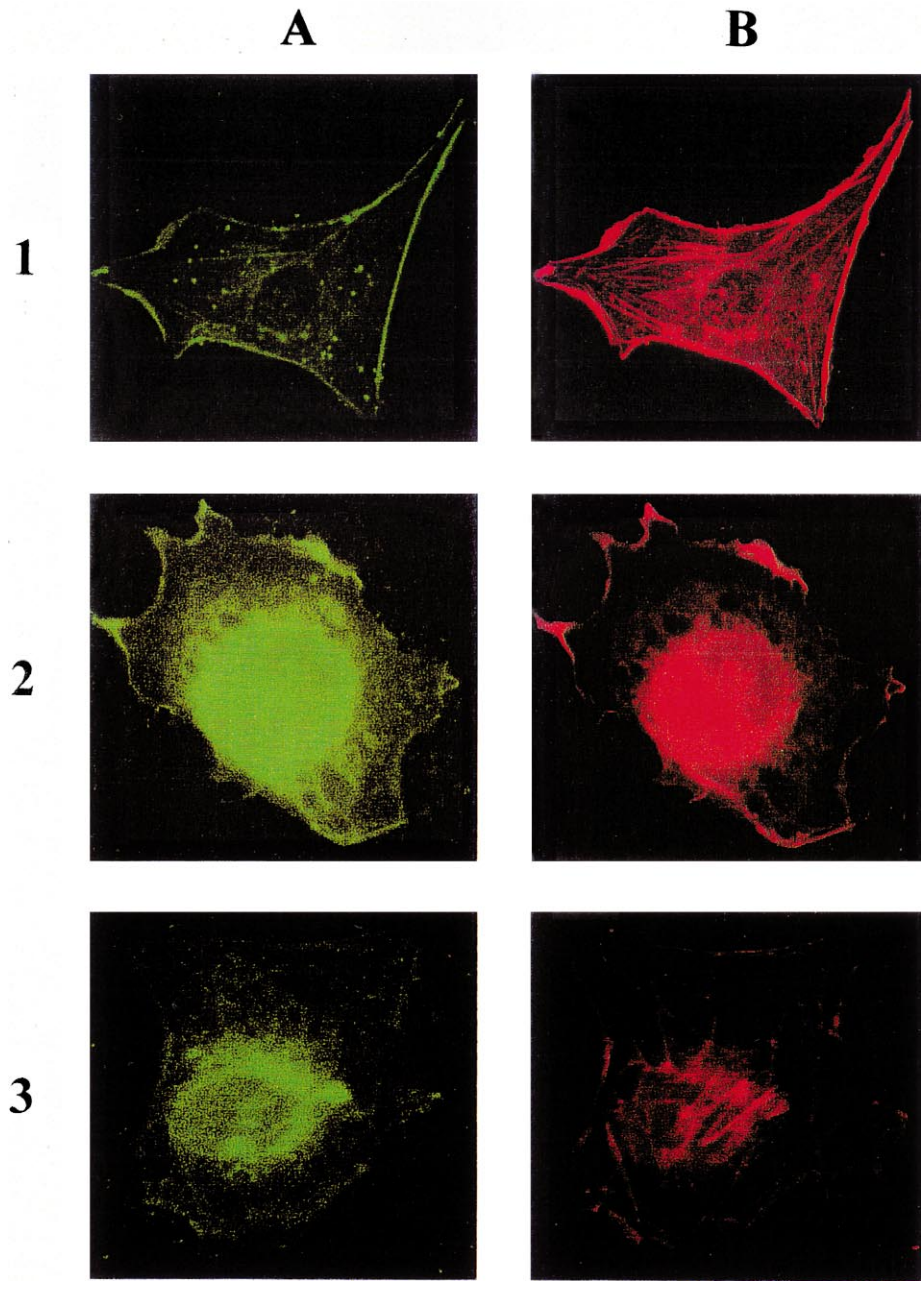

Fig. 3. Effect of cerivastatin on localization of RhoA and on actin stress fibers after a 18 h incubation. Panels 1A,B: bFGF 25 ng/ml, panels 2A,B: cerivastatin 10 ng/ml+bFGF 25 ng/ml, panels 3A,B: cerivastatin 25 ng/ml+bFGF 25 ng/ml.

Then, 10 μl of each supernatant were loaded on a 7.5% polyacrylamide gel containing 10% SDS and 1 mg/ml gelatin under non-reducing conditions and then subjected to electrophoresis. Gels were then washed in 2.5% Triton X-100 for 1 h at room temperature in order to remove SDS. Gelatinase activity was revealed by its gelatinolic activity after an overnight incubation at 37°C in fresh developing buffer containing 50 mM Tris–HCl, 5 mM $CaCl_2$, pH 7.6. The gel was then stained with Coomassie brilliant blue R-250 solution (0.25%). Gelatinolic activity was evidenced as clear bands against the blue background of stained gelatin.

2.7. Statistical analysis

Significant values were determined using a two-tailed non-parametric Mann–Whitney test using the InStat software (Sigma). The results are expressed as mean value ± standard error of the mean (S.E.M.). $P < 0.05$ was considered as significant.

3. Results and discussion

Cerivastatin has been demonstrated to inhibit both migration and proliferation of smooth muscle cells [7]. However, its effect on microvascular endothelial cells has not yet been explored. In this work, we demonstrated that cerivastatin induced a dose-dependent decrease in endothelial cell migration in two different models.

3.1. Cerivastatin inhibited the endothelial cell migration in a transwell system

Cerivastatin (10 and 25 ng/ml) induced a significant inhibition of OSM-, bFGF- and VEGF-stimulated endothelial cell

migration from the upper chamber to the lower one through the membrane (Table 1). Moreover, the inhibitory effect of cerivastatin on HMEC-1 cell migration was fully reversible by co-incubation with MVA or GGPP but not with FPP (Table 1). Cerivastatin did not inhibit the migration of unstimulated endothelial cells, suggesting that cerivastatin has only an inhibitory effect on endothelial cells activated by angiogenic factors. This result indicates that cerivastatin could suppress the angiogenic factors-stimulated cell locomotion in responding to chemotaxis agents. Moreover, cerivastatin did not induce any toxic effect as shown by the absence of trypan blue incorporation into the cells. These results indicate that cerivastatin could suppress the angiogenic factors-stimulated cell locomotion in responding to chemotaxis agents and this effect is mainly related to the inhibition of GGPP synthesis.

3.2. Cerivastatin inhibited the endothelial cell migration in wound healing assay

In the wound healing assay, cerivastatin (25 ng/ml) inhibited cell migration in both stimulated (Fig. 1, panels 2B,C) or unstimulated (Fig. 1, panel 2A) endothelial cells in a dose-dependent manner (data not shown for 10 ng/ml of cerivastatin). Co-treatment of cerivastatin with MVA (Fig. 1, panels 4A–C) or GGPP (Fig. 1, panels 8A–C) reversed this inhibitory effect while FPP did not (Fig. 1, panels 6A–C). Similar results were demonstrated on VEGF-stimulated cells (data not shown). These results confirm that the inhibition of cell migration induced by cerivastatin is mainly due to the inhibition of GGPP synthesis.

3.3. Cerivastatin inhibited the capillary tube formation

After 4 days of culture in fibrin matrix, the formation of tube like structure was observed under phase contrast microscopy. When cerivastatin was added to the fibrin matrix, a low dose of this drug (10 ng/ml) was sufficient to abolish the tube formation in the absence or in the presence of angiogenic factors (Fig. 2, panels 2A–D). MVA and GGPP reversed the inhibitory effect of cerivastatin (25 ng/ml) on capillary tube formation (Fig. 2, panels 4A–D and 6A–D, respectively). FPP also reversed the effect of cerivastatin but only partially (Fig. 2, panels 5A–D). Same reversions were observed in presence of 10 ng/ml of cerivastatin (data not shown). Control done without cerivastatin showed that MVA, FPP and GGPP alone did not modify the capillary tube formation (data not shown).

Table 2
Effect of a 18 h incubation with cerivastatin on the fluorescence profile of RhoA and actin associated to cell membrane of endothelial cells

	RhoA		Actin	
	Mean	S.E.M.	Mean	S.E.M.
Control	126.82	13.8	254.55	3.49
Cerivastatin 10 ng/ml	83.65	7.9*	126.35	7.75***
Cerivastatin 25 ng/ml	31.26	5.17**	51.69	6.61***

bFGF-stimulated HMEC-1 were incubated for 18 h with cerivastatin at indicated concentrations. Fluorescence profile of RhoA and actin associated to cell membrane was then performed by the computer-assisted image analysis of the confocal microscope. Results are expressed as the mean of fluorescence (arbitrary fluorescence unity) ± S.E.M. (*$P < 0.05$; **$P < 0.001$ for RhoA as compared with cerivastatin-untreated cells, ***$P < 0.001$ for actin as compared with cerivastatin-untreated cells, $n = 4$).

This observation showing that GGPP elicited a greater reversion of cerivastatin effect than FPP, indicates that the inhibitory effect of cerivastatin on angiogenesis is mainly due to the inhibition of GGPP synthesis, as already noted for cell migration.

3.4. Cerivastatin induced a delocalization of RhoA from cell membrane and actin depolymerization

As indicated above, all results indicate that the effect of cerivastatin is related to the inhibition of isoprenoids biosynthesis and mostly GGPP. Therefore, as geranylation of RhoA is implicated in cell membrane translocation and cell locomotion, we investigated the RhoA distribution on bFGF-stimulated endothelial cells. Confocal microscopy assay was performed to localize RhoA in the cell compartment. In absence of cerivastatin, RhoA was present at the membrane periphery and at the lamellipodia extensions and occurred in stress fibers (Fig. 3, panel A1). After a 18 h treatment with 10 ng/ml of cerivastatin, RhoA remained largely diffused in the cytoplasm mainly in the perinuclear region (Fig. 3, panel A2). Parallel to the delocalization of RhoA from cell membrane, cerivastatin completely inhibited the formation of actin filaments (Fig. 3, panel 2B). Neither organized actin filaments nor focal adhesion points were detected after a 18 h treatment with 25 ng/ml cerivastatin (Fig. 3, panels 3A,B). As shown on Table 2, the study of the fluorescence profile evaluated on cell membrane showed that cerivastatin dose-dependently and significantly decreased cell membrane-associated RhoA and actin. It was checked that in the absence of the first antibody, no fluorescence was detected as control (data not shown).

Therefore, we have demonstrated that cerivastatin induced a delocalization of RhoA from cell membrane to the cytoplasm and this effect led to the disruption of skeleton actin stress fibers. This was associated with cell rounding. As the RhoA GTPases have been shown to play a key role on cell migration and invasion [21], the inhibition of endothelial cell migration and tube formation induced by cerivastatin could be due to the inhibition of RhoA translocation from cytoplasm to the cell membrane.

3.5. Cerivastatin decreased the secretion of MMP-2

Zymography showed that after a 24 h incubation with cerivastatin, the band corresponding to MMP-2 (molecular weight 72 000 Da) was dose-dependently reduced. The activity of this MMP was remarkably inhibited from 10 ng/ml of cerivastatin (Fig. 4A, lane 3). At 25 ng/ml of cerivastatin, MMP-2 activity was completely inhibited (Fig. 4A, lane 4). Parallel to the decrease of MMP-2 activity, RT-PCR assay revealed that incubation of endothelial cells for 6 h with cerivastatin induced a 50% decrease of mRNA intensity at 10 ng/ml (Fig. 4B, lane 3) and 62% decrease at 25 ng/ml (Fig. 4B, lane 4).

Co-incubation of endothelial cells with cerivastatin (25 ng/ml) and either MVA or FPP reversed the cerivastatin-induced inhibition of MMP-2 activity as shown by zymography analysis (Fig. 4D, lane 4 for MVA, lane 6 for FPP) while GGPP did not (Fig. 4D, lane 8). Therefore, the dose-dependent inhibition of MMP-2 secretion induced by cerivastatin on endothelial cells could be related to the inhibition of the Ras pathway secondary to the inhibition of FPP formation. In fact, it has been recently demonstrated that LPS-activated MMP-2 expression on endothelial cells was mediated through

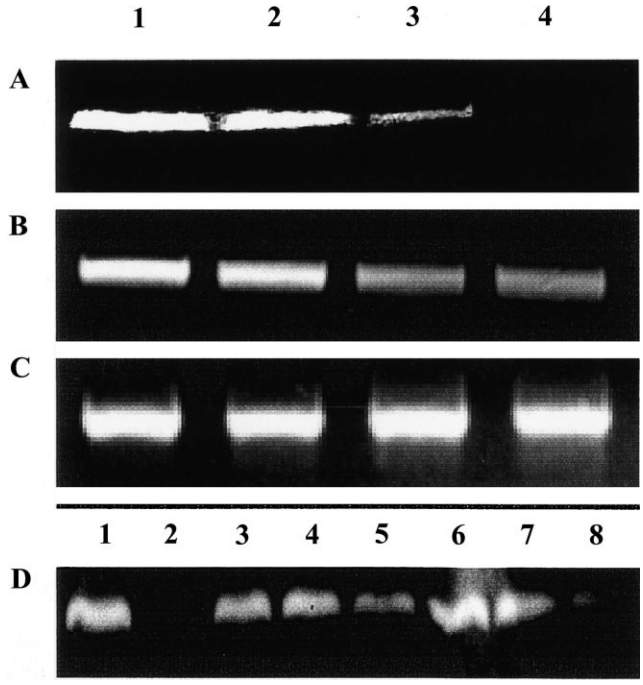

Fig. 4. Inhibitory effect induced by cerivastatin on mRNA expression and protein activity of MMP-2. Effect of MVA, FPP and GGPP on cerivastatin-induced inhibition of MMP-2 activity. A: Zymography of MMP-2 protein activity; lane 1, control; lane 2, cerivastatin 5 ng/ml; lane 3, cerivastatin 10 ng/ml; lane 4, cerivastatin 25 ng/ml. B: MMP-2 mRNA expression after 6 h incubation; lane 1, control; lane 2, cerivastatin 5 ng/ml; lane 3, cerivastatin 10 ng/ml; lane 4, cerivastatin 25 ng/ml. C: β-Actin mRNA expression after 6 h incubation; lane 1, control; lane 2, cerivastatin 5 ng/ml; lane 3, cerivastatin 10 ng/ml; lane 4, cerivastatin 25 ng/ml. D: Zymography of MMP-2 protein activity; lane 1, control; lane 2, cerivastatin 25 ng/ml; lane 3, MVA 100 μM; lane 4, MVA 100 μM+cerivastatin 25 ng/ml; lane 5, FPP 10 μM; lane 6, FPP 10 μM+cerivastatin 25 ng/ml; lane 7, GGPP 10 μM; lane 8, GGPP 10 μM+cerivastatin 25 ng/ml.

an NF-κB pathway [22], which was activated by the translocation of Ras [11].

All these results demonstrate that cerivastatin, an inhibitor of HMG-CoA reductase, induces an inhibition of angiogenesis. This inhibition could explain, at least in part, the protective effect of the drug against atherothrombotic events which were higher than that expected by the cholesterol decrease. Indeed, angiogenesis is involved in plaque progression and fragilization leading to plaque rupture and adverse clinical outcome due to occlusive thrombi formation.

Our results are in contrast with the recently published data of Kureishi et al. [23], which reported that statins promote angiogenesis, a phenomenon attributed to Akt activation. The protein kinase Akt, a downstream effector of the PI-3 kinase, has been clearly demonstrated to promote angiogenesis by inducing actin reorganization and membrane ruffling [24–26]. The conclusion of Kureishi et al. [23] does not match our observations which show that cerivastatin strongly inhibits actin stress fibers organization and consequently endothelial cell migration. In addition, as Akt could be activated through Ras activation (an upstream activator of PI-3 kinase), this Akt pathway is not thought to be activated by statins treatment because of their inhibiting effect on Ras and RhoA activation [27].

This discrepancy could be due to the difference of the endothelial cell origin as we used microcapillary endothelial cells whereas these authors used human umbilical vascular endothelial cells or bovine aortic endothelial cells both representatives of macrovasculature. The anti-angiogenic effect of cerivastatin described in this study was also confirmed using another endothelial cell from microvasculature of bone marrow origin (human bone marrow endothelial cell; results not shown).

In conclusion, in our experimental conditions, cerivastatin strongly inhibits endothelial cell locomotion and capillary tube formation, indicating that cerivastatin could be considered as an anti-angiogenic substance. Its inhibitory effect was reversed by MVA and GGPP indicating that it was related to the inhibition of GGPP formation. As RhoA activation is dependent on geranylgeranylation, we suggest that the inhibitory effect of cerivastatin on endothelial cell migration is mainly related to the inhibition of RhoA activation. This is in good accordance with the cerivastatin-induced translocation of RhoA from cell membrane to the cytoplasm. In addition, FPP partially reversed the anti-angiogenic activity of cerivastatin, probably by reversing the inhibition of MMP-2 secretion.

Currently, statins are among the most commonly prescribed drugs in patients with vascular risk. Our results suggest that anti-angiogenic effects of statins should be considered for inhibiting atherosclerosis as expected but might also inhibit tumor progression. This has been supported by clinical studies which have demonstrated that statin treatment reduced the incidence of cancers [28].

Acknowledgements: We are grateful to Dr. Bischoff (Bayer, Germany), Dr. Chartier and Dr. Barouki (Bayer, France) who provided cerivastatin and for their helpful advice. This work was supported by grants from le Groupement des Entreprises Françaises dans la Lutte contre le Cancer (GEFLUC), l'Association Régionale pour l'Enseignement et la Recherche Scientifique technologique (ARERS), La Ligue contre le Cancer de la Seine-maritime et de l'Eure and la Région Haute Normandie. L.V. is a recipient of a fellowship from the GEFLUC. The authors thank Elisabeth Legrand for her technical assistance in the realization of this work and Richard Meideros for his valuable editorial assistance.

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